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Journal of Microbiological Methods 50 (2002) 131–139

Journal of Microbiological Methods

www.elsevier.com/locate/jmicmeth

A comparison of five methods for extraction of bacterial DNA from human faecal samples

Alexandra L. McOrist*, Michelle Jackson, Anthony R. Bird

CSIRO Health Sciences and Nutrition, PO Box 10041, Adelaide BC, SA 5000, Australia

Received 18 October 2001; received in revised form 15 January 2002; accepted 17 January 2002

Abstract

The purity of DNA extracted from faecal samples is a key issue in the sensitivity and usefulness of biological analyses such as PCR for infectious pathogens and non-pathogens. We have compared the relative efficacy of extraction of bacterial DNA (both Gram negative and positive origin) from faeces using four commercial kits (FastDNA® kit, Bio 101; Nucleospin® C+T kit, Macherey-Nagal; Quantum Prep® Aquapure Genomic DNA isolation kit, Bio-Rad; QIAamp® DNA stool mini kit, Qiagen) and a non-commercial guanidium isothiocyanate/silica matrix method. Human faecal samples were spiked with additional known concentrations of *Lactobacillus acidophilus* or *Bacteroides uniformis*, the DNA was then extracted by each of the five methods, and tested in genus-specific PCRs. The Nucleospin® method was the most sensitive procedure for the extraction of DNA from a pure bacterial culture of Gram-positive *L. acidophilus* (10^4 bacteria/PCR), and QIAamp® and the guanidium method were most sensitive for cultures of Gram-negative *B. uniformis* (10^3 bacteria/PCR). However, for faecal samples, the QIAamp® kit was the most effective extraction method and led to the detection of bacterial DNA over the greatest range of spike concentrations for both *B. uniformis* and *L. acidophilus* in primary PCR reactions. A difference in extraction efficacy was observed between faecal samples from different individuals. The use of appropriate DNA extraction kits or methods is critical for successful and valid PCR studies on clinical, experimental or environmental samples and we recommend that DNA extraction techniques are carefully selected with particular regard to the specimen type.

Keywords: *Bacteroides uniformis*; DNA extraction; Faecal samples; *Lactobacillus acidophilus*; PCR

1. Introduction

Bacteria play important roles in the health of the gastrointestinal tract including the fermentation of potential energy sources, production of short chain fatty acids, and activation or deactivation of carcinogens. There is increasing interest in how diet influences bacterial populations in either a positive or negative fashion (Topping and Clifton, 2001) and more sophisticated methods of analysis are being used to identify species and enumerate bacteria from biological samples. The molecular analysis of clinical samples for microbes can offer various advantages over cultural methods, including detection of a wider range of target organisms, and greater sensitivity and specificity. Compared to blood or other clinical samples, the complex microbial flora, variable consistency and variable endogenous and dietary components of faeces make DNA extraction particularly difficult. The purity of DNA extracted from this heterogeneous material is a

* Corresponding author. Tel.: +61-8-8303-8949.
E-mail address: sandi.mcorist@csiro.au (A.L. McOrist).

0167-7012/02/$ - see front matter
PII: S0167-7012(02)00018-0

key issue in the sensitivity and usefulness of further analyses, such as PCR analysis for infectious pathogens. Our studies have focused on detecting the levels of *Lactobacillus* and *Bacteroides*, Gram-positive and Gram-negative, respectively, in faeces by PCR analysis. These genera are common inhabitants of the human colon.

Time constraints may make traditional phenol-chloroform extraction of bacterial DNA impractical as additional clean-up procedures may be required to remove carry-over phenols which inhibit the polymerase chain reaction. Other methods have been developed, usually involving lysis of bacteria and subsequent binding of released DNA onto a solid matrix, followed by washing and elution of the relevant components. An example of these methods is the addition of guanidium isothiocyanate to faeces for lysis of bacterial cells, with a silica matrix to hold DNA allowing the wash removal of proteins and final elution of DNA (Boom et al., 1990). This method has proved useful for gathering faecal or digesta DNA for PCR analysis of enteric bacteria, including those incapable of cell-free growth (Jones et al., 1993). Largely because of high labour costs and time constraints in processing numerous clinical samples, commercial kits have been developed and are becoming used widely for DNA extractions. Some of these have been specifically developed for faecal analysis, as faeces contain several inhibitors of PCR reactions, such as bile salts, haemoglobin degradation products and complex polysaccharides (Monteiro et al., 1997; Widjojoatmodjo et al., 1992). However, the relative efficiency and effectiveness of these extraction methods has not been fully explored. Therefore, we compared the relative efficacy of extraction of bacterial DNA (from both Gram-negative and -positive species) from faeces using four commercial kits and by the Boom method, followed by specific PCR analysis of the DNA extract.

2. Material and methods

2.1. Human faecal samples

A fresh faecal specimen was obtained from one adult male (H1) and one adult female (H2), who had been on similar diets for 12 weeks as part of a weight control study with CSIRO Health Sciences and Nutrition. Ethics approval was given by the Human Ethics Committee of Commonwealth Scientific and Industrial Research Organisation Health Sciences and Nutrition and complied with the Helsinki Declaration of 1975 as revised in 1983. Total daily energy intake of the subject was restricted to 6000 kJ in order to elicit a modest weight loss. Neither volunteer had a history of gastrointestinal disease or antibiotic use prior to the study. The faecal specimens (10 g) from each subject were suspended in 90 ml peptone water and homogenised in a stomacher apparatus. Samples were vigorously vortexed for 1 min, then centrifuged at $65 \times g$ to deposit any remaining solid matter. The supernatant was centrifuged at $12{,}000 \times g$ for 5 min at 4 °C and the pellet washed twice in 25 ml of 0.01 M Tris–HCl pH 8.0, containing 1 mM EDTA (TE buffer). The final pellet from each human faecal specimen was resuspended in 100 ml of TE buffer and 0.5 ml aliquots prepared. The numbers of *Lactobacillus* spp. and total anaerobes in each faecal specimen were determined by routine culture and dilution counts (Messer et al., 2000).

2.2. Test bacteria

The origin and identity of the various bacteria and bacterial DNA isolates used in this study are given in Table 1. The *L. acidophilus* and *B. uniformis* isolates used for spiking of faecal samples were cultured on Rogosa and Colombia blood agar, respectively, under standard conditions (Batt, 2000; Flint and Stewart, 2000). Bacteria were harvested from agar plates and serial 10-fold dilutions prepared in TE buffer. The numbers of *L. acidophilus* and *B. uniformis* in each harvest, and added to faecal samples, were determined by routine culture and dilution counts (Messer et al., 2000). A dilution series was prepared in TE buffer to give approximate final genome equivalents per PCR ranging from 10^7 to 1 bacterium. DNA was prepared from 0.5 ml of each dilution by various extraction methods to represent pure culture DNA. A 0.5-ml sample of each fresh dilution was added to 0.5 ml of faecal samples from H1 and H2, and the DNA was extracted immediately by each method.

2.3. DNA extraction

The following four commercial DNA extraction kits were used on faecal specimens and bacterial

Table 1
PCR results from a panel of common enteric bacteria using primers designed for the PCR amplification of *Lactobacillus* and *Bacteroides* species

Bacterium	Origin	Primary *Lactobacillus* PCR	Nested *Lactobacillus* PCR	Primary *Bacteroides* PCR	Nested *Bacteroides* PCR
Bacteroides fragilis	UE	–	–	+	+
Bacteroides vulgatus	UE	–	–	+	+
Bacteroides caccae	UE	–	–	+	+
Bacteroides stercoris	UE	–	–	+	+
Bacteroides uniformis	UE	–	–	+	+
Bacteroides thetaiotaomicrom	UE	–	–	+	+
Bacteroides ovatus	UE	–	–	+	+
Bacteroides distasonis	UE	–	–	+	+
Bacteroides merdae	UE	–	–	+	+
Lactobacillus acidophilus	GB	+	+	–	–
Lactobacillus casei	GB	+	+	–	–
Lactobacillus plantarum	UE	+	+	–	–
Bifidobacterium bifidum	GB	–	–	–	[a]
Bifidobacterium longum	GB	–	–	–	–
Desulfovibrio desulfuricans	UM	–	–	–	–
Bilophila wadsworthia	UM	–	–	–	–
Lawsonia intracellularis	UM	–	–	–	–
Listeria monocytogenes	UA	–	–	–	–
Shigella flexineri	UA	–	–	–	–
Escherichia coli B41	UE	–	–	–	–
Escherichia coli B44	UE	–	–	–	–
Staphylococcus aureus	UE	–	–	–	–
Streptococcus dysgalactacia	UE	–	–	–	–
Streptococcus faecalis	UE	–	–	–	–
Streptococcus salivarius	UE	–	–	–	–
Streptococcus viridans	UE	–	–	–	[a]
Streptococcus zooepidemicus	UE	–	–	–	–
Campylobacter jejuni	UE	–	–	–	–
Citrobacter freundii	UE	–	–	–	–
Proprionibacterium acnes	UE	–	–	–	–

+ = PCR product, – = no PCR product. UE = University of Edinburgh, UM = University of Minnesota, UA = University of Adelaide, GB = Gist-Brocade.

[a] Very weak product band observed.

dilution and culture series, according to the manufacturers' instructions:

1. FastDNA® kit (Bio 101, Carlsbad, CA, USA) using 0.25-in. sphere and garnet mix and two 30-s pulses at speed 5 of the FastPrep™ Mini bead beating instrument (Bio 101).
2. Nucleospin® C+T kit[1] (Macherey-Nagal, Germany).
3. Quantum Prep® Aquapure Genomic DNA isolation kit (Bio-Rad, Hercules, CA, USA).
4. QIAamp® DNA stool minikit (Qiagen, Germany).

[1] Not used on faeces H2.

The four manufacturers did not supply complete information and composition of some of the ingredients of their commercial kit due to the proprietary nature of some kit components. Technical information supplied with kits 1, 2 and 4 indicated that lysis buffers were high-strength chaotropic guanidium salts and detergents, with washing buffers consisting of initial low strength chaotropic salts, and Tris/alcohol/acid buffers for DNA elution. Technical information supplied with kit 3 indicated that lysis buffers contain detergent, proteins are removed by precipitation, and DNA extracted in isopropanol. Kit 4 included a commercial polysaccharide mixture for the described purpose of removing PCR inhibitors of faecal origin; this was added to the lysis buffer.

In addition to DNA extraction with proprietary kits, each sample was processed using the Boom method (referred to as method 5 in subsequent sections) (Boom et al., 1990) whereby faecal and bacterial series samples were centrifuged at 12,000 × *g* for 3 min and the bacterial pellet resuspended in 5 M guanidium isothiocyanate in 50 mM Tris buffer (pH 6.4, 22 mM EDTA, 0.65% Triton X-100). After 1-h incubation at room temperature, 15 μl of 20% w/v diatomaceous earth (DE) in 0.17% HCl was added and incubated on ice for 5 min. Samples were centrifuged at 12,000 × *g* for 20 s, and the pellet washed thrice in 5.5 M guanidium thiocyanate in 50 mM Tris buffer pH 6.4, twice in 70% ethanol, the pellet dried at 60 °C for 15 min, and DNA was eluted from the DE in 200 μl TE buffer at 48 °C. The final volume for each DNA preparation was 200 μl.

2.4. PCR primers and methods

Primers for the genera *Lactobacillus* and *Bacteroides* were designed by processing the 16S rDNA sequences from six species of *Bacteroides* (Genbank accession numbers L16486 *B. uniformis*, M58763 *B. thetaiotaomicron*, X83952 *B. ovatus*, X83943 *B. fragilis*, M58762 *B. vulgatus*, M86695 *B. distasonis*) and seven species of *Lactobacilli* [Genbank accession numbers X61138 *L. acidophilus*, M99704 *L. johnsonii*, M58810 *L. brevis*, M58819 *L. fermentum*, M58827 *L. plantarum*, L23507 *L. reuteri*, and M59054 for *L. salivarius*] separately through GCG software (Whitehead Institute, USA) "PILEUP", "CLUSTAL" and "PRIMER" software accessed from the Human Genome Mapping Project (HGMP), Cambridge, UK. Selected genus primers, nested primers and the projected PCR product lengths are shown in Table 2. The specificity of the design was examined by the processing of the primer sequences through "FIND PATTERN" software, which compared the DNA homology of these *Lactobacillus* and *Bacteroides* primer sequences to that of all the other DNA sequences contained in the EMBO databases.

PCR incorporating DNA samples from cultured strains of *Lactobacillus* spp. and *Bacteroides* spp., and taxonomically related and unrelated enteric bacteria was performed to further confirm the specificity of each PCR. The conditions of the amplification reactions were 1 cycle of 94 °C for 4 min, 60 °C for 1 min, 72 °C for 1 min, 28 cycles at the same temperatures, but 1 min per step, with a final extension step of 4 min for the 72 °C. The final reaction mixture (50 μl total volume) for each reaction was × 1 reaction buffer A (Promega, USA), 200 mM dNTPs, 1 U *Taq* polymerase (Promega, USA), 100 nmol of each primer, 3% (vol/vol) DMSO, 1.5 mM $MgCl_2$ and 2 μl of the final eluate DNA preparation. Each nested PCR was performed using 2 μl of the primary PCR reaction as the DNA sample under the same reaction conditions, but with the nested primer sets. Dilutions, 1:10 and 1:100, of each DNA extraction were prepared in TE buffer and 2 μl tested in *Lactobacillus* or *Bacteroides* PCR. Albumin was not added to any PCR mixture.

Portions of H1 and H2 faecal samples spiked with dilution series of *L. acidophilus* or *B. uniformis* were subjected to each DNA extraction method, and the final DNA eluate incorporated into separate direct (and nested) PCR for *Lactobacillus* and *Bacteroides*

Table 2
Primer sequences for the PCR amplification of *Lactobacillus* and *Bacteroides* species

Genus		Sequence 5′–3′	Product length
Lactobacillus	Sense	TGGAAACAGGTGCTAATACCG	247 bp
	Antisense	CCATTGTGGAAGATTCCC	
Lactobacillus nest	Sense	As for primary PCR	230 bp
	Antisense	CCCTACTGCTGCCTCCCGTAG	
Bacteroides	Sense	TCCACCTGGGGAGTACGCCG	220 bp
	Antisense	TATGGCACTTAAGCCGACACC	
Bacteroides nest	Sense	GGAGTACGCCGGCAACGGTG	207 bp
	Antisense	CTTAAGCCGACACCTCACGG	

Table 3
Primary [1°] and secondary nested [2°] PCR products from DNA extracted from pure bacterial culture and faecal samples, H1 and H2, spiked with known concentrations [1–10^7] of (A) *L. acidophilus* or (B) *B. uniformis* prior to DNA extraction

Method	1		2		3		4		5	
	Fast DNA®		Nucleospin®		Aquapure		QIAamp®		Boom et al.	
PCR	1°	2°	1°	2°	1°	2°	1°	2°	1°	2°
(A) Lactobacillus acidophilus										
Pure *L. acidophilus*										
10^7	+	+	++	+	++	+	+	+	+	+
10^6	–	+	++	+	–	+	++	+	+	+
10^4	–	+	+	+	–	–	+	+	–	+
10^3	–	+	+	+	–	–	–	+	–	+
10^2	–	–	–	+	–	–	+	+	–	+
10	–	–	–	+	–	–	–	+	–	+
1	–	+	–	+	–	–	–	+	–	+
Faeces H1										
10^7	–	+	–	+	–	+++	++	+++	+	+++
10^6	–	+	–	+	–	++	+	+++	–	–
10^4	–	+	–	++	–	++	+	+++	–	–
10^3	–	+	–	+	–	++	+	+++	–	–
10^2	–	+	–	+	–	++	+	+++	–	–
10	–	+	–	+	–	++	+	+++	–	–
1	–	+	–	+	–	++	++	+++	–	–
Faeces H2										
10^7	+	+++	+	+++	++	++++	++	+++	++	++++
10^6	–	+++	–	++	++	++++	++	+++	–	+++
10^4	–	++	–	+	–	+++	+	+++	–	+++
10^3	–	++	–	+++	+	+++	+	+++	–	+++
10^2	–	++	–	+++	–	+++	+	+++	–	++
10	–	++	–	++	–	+++	–	+++	–	+++
1	–	++	–	++	–	+++	+	+++	–	++
(B) Bacteroides uniformis										
Pure *B. uniformis*										
10^7	+++	++	+++	++++	+++	+++	+++	++++	++	++++
10^6	+	++	+++	+++++	++	+++	+++	++++	++	++++
10^4	–	++	+	++++	+	+++	+	++++	+	++++
10^3	–	+	–	++++	–	++	+	++++	+	+++
10^2	–	++	–	+++	–	++	–	++++	–	+
10	–	++	–	+	–	++	–	++	–	+
1	–	+	–	++	–	+	–	+++	–	+
Faeces H1										
10^7	–	++	–	++	++	++++	++++	+++++	–	+++
10^6	–	++	–	+	+	+++	+++	++++	–	+++
10^4	–	++	–	++	–	+++	+++	++++	–	+++
10^3	–	+	–	–	+	+++	+++	++++	–	+++
10^2	–	++	–	–	–	++	+++	++++	–	+++
10	–	++	–	–	+	+++	+++	++++	–	+++
1	–	+	–	–	–	++	+++	++++	–	+++

(continued on next page)

Table 3 (*continued*)

Method	1		2		3		4		5	
	Fast DNA®		Nucleospin®		Aquapure		QIAamp®		Boom et al.	
PCR	1°	2°	1°	2°	1°	2°	1°	2°	1°	2°
(B) Bacteroides uniformis										
Faeces H2										
10^7	++	++++	ND	ND	+++	++++	++++	++++	++	+++
10^6	+	++++	ND	ND	+++	++++	++++	++++	++	+++
10^4	+	++++	ND	ND	+++	++++	++++	++++	+	+++
10^3	+	++++	ND	ND	+++	++++	++++	++++	++	+++
10^2	+	+++	ND	ND	++	+++	+++	++++	+	+++
10	+	+++	ND	ND	++	+++	+++	++++	+	+++
1	+	+++	ND	ND	+++	+++	+++	++++	+	+++

The density of PCR products on agarose gels was determined using Quantity One software (Bio-Rad) and expressed as integrated density units (IDU). Results were classified as follows −=PCR negative, +=1–99 IDU, ++=100–249 IDU, +++=250–499 IDU, ++++=500–749 IDU, +++++=750–999 IDU, and ND=not done.

spp. PCR products were visualised by electrophoresis (100 V for 2 h) through 1.8% Metaphor agarose gel (FMC BioProducts, Rockland ME, USA) and stained with ethidium bromide. Images of each gel were captured in a Kodak D256 digital camera and transferred to a computer. Computer-assisted densitometry of each PCR product band was performed using Quantity 1 software (Bio-Rad). The minimum level of repeatability was calculated by running DNA samples from duplicated amplifications of each DNA extract. To limit the possible problems of repeatability and reproducibility, the samples to be compared were processed concurrently.

DNA extracts from selected faecal samples and from extracts of pure cultures of *Lactobacillus* spp. and *Bacteroides* spp. were incorporated into the test PCR analyses and the resultant PCR product subjected to single-strand conformational polymorphism analysis (SSCP, Orita et al., 1989) to confirm the identity of the PCR products from faecal sources.

3. Results

3.1. Human faecal samples

The *Lactobacillus* spp. colony counts from Rogosa agar culture for the original H1 and H2 faecal samples were 8.94 and <3 $\log_{10}$ cfu/g, respectively. The total anaerobe colony counts from Colombia agar culture for H1 and H2 were 9.90 and 9.78 $\log_{10}$ cfu/g, respectively. If *Bacteroides* spp. represent approximately 30% of all culturable faecal isolates (Salyers, 1984), the number of *Bacteroides* spp. in samples H1 and H2 would be estimated at 9.38 and 9.26 $\log_{10}$ cfu/g, respectively.

3.2. PCR of Lactobacillus and Bacteroides

PCR designed for *Lactobacillus* and *Bacteroides* consistently produced products of the predicted molecular size when control positive samples were used for DNA template, with no detectable products produced in DNA templates prepared from other bacteria listed in Table 1. A BLAST search indicated that the primary *Lactobacillus* primer sequences were specific for 40 *Lactobacillus*, 5 *Pediococcus*, 4 *Leuconostoc* and 1 *Lactococcus* spp. All of these bacteria belong to the *Lactobacillaceae* family except *Lactococcus*, which is a member of the *Streptococcaceae* family. A similar search for the primary *Bacteroides* primers indicated that they were specific for 15 *Bacteroides*, 4 *Porphyromonas*, 2 *Prevotella* species, and *Weeksella virosa*. All are members of the *Bacteroidaceae* family except for *Weeksella*, which is a member of the *Flavobacteriaceae* family.

3.3. Comparison of DNA extraction methods

Five different DNA extraction methods were examined to ascertain their relative effectiveness for extracting bacterial DNA from faeces. All of the meth-

ods extracted bacterial DNA successfully from pure cultures, as demonstrated by successful genus-specific PCR. Of the five methods, kit 2 was the most effective for the detection of DNA prepared from pure bacterial culture of Gram-positive *L. acidophilus* (10^4 bacteria/PCR), and kit 4 and the Boom method were most effective for Gram-negative *B. uniformis* (10^3 bacteria/PCR) (see Table 3). All these methods were less effective in extracting bacterial DNA from faeces than from pure culture. Densitometry readings derived from bacterial PCR performed on each faecal sample spiked with serial dilutions of bacteria are shown in Table 3. For faecal samples, kit 4 (QIAamp®) was the most effective extraction method and led to the detection of bacterial DNA over the greatest range of spike concentrations for both *B. uniformis* and *L. acidophilus*. Dilution of template DNA extracted from both pure culture and faecal samples did not improve the amplicon production in *Lactobacillus* or *Bacteroides* PCR.

A difference in PCR outcome was observed between faecal samples. For sample H2, DNA extraction was successful for samples containing the 10^7 spike of *L. acidophilus* in all five methods. However, only kit 4 and method 5 resulted in PCR product from sample H1 at the same spike concentration. A similar result was observed for samples spiked with *Bacteroides* spp., except that only kits 3 and 4 were successful with faecal sample H1. Some DNA extractions from faeces spiked with bacterial DNA failed to produce PCR product from sample H1, possibly due to the presence of inhibitors or ineffective DNA extraction. These include kits 1, 2 and 3 for *Lactobacillus* spp., and kits 1, 2 and method 5 for *Bacteroides* spp. Where sufficient PCR product was available SSCP analysis demonstrated identity between pure culture patterns for *L. acidophilus* or *B. uniformis* and those derived from faecal samples (data not shown).

In our hands, repetition of testing of each DNA extract in the nested PCRs confirmed the superiority of kit 4, with consistently higher levels of nested PCR product from each faecal sample over the range of spike concentrations (see Table 3). Product was obtained from samples using nested PCR that failed to produce product in the primary reaction, albeit at a greatly reduced concentration than that produced by DNA extracted using kit 4.

4. Discussion

Our findings indicate that, within the limitations of the study, the use of the kit 4, "QIAamp® DNA stool minikit" resulted in the extraction of bacterial DNA from faeces which resulted in superior downstream performance in PCR compared to the other methods applied under similar conditions. To test the quality of extracted DNA, we designed genus-specific PCR primers to amplify 16S rDNA from *Lactobacillus* or *Bacteroides* genera. These genera are commonly present in faeces and represent both Gram-positive and -negative bacterial phenotypes. *Bacteroides* spp. are thought to represent 30% of all anaerobic bacteria in the colon (Salyers, 1984). Fresh (non-sterilised) faeces were considered to provide the most realistic clinical material for comparative purposes, even though the exact number of indigenous bacteria could not be standardised for each sample. The purpose of the addition of known bacterial colony forming units to each test faecal sample was to provide a minimum and a serially increasing number of known bacteria to assess the relative performance of the various DNA extraction methods. Faecal samples were also likely to contain indigenous species of the genus. These extractions provided DNA template that is useful for subsequent PCR analysis.

We have shown that different extraction techniques result in variable sensitivity of PCR detection for DNA of bacterial species in human faecal samples. Importantly, these relative sensitivities could not have been extrapolated from the results obtained for DNA extractions performed directly from pure cultures. Other studies have shown that the sensitivity of PCR is also dependent on primer design and efficiency and can vary from 2 to 10,000 cells per PCR when using pure culture DNA (Wang et al., 1996). It should be noted that our experiments were not designed to give truly quantitative data, but to illustrate qualitative differences achieved using different DNA extraction methods on biological samples. We did not achieve a particularly high degree of sensitivity with pure culture DNA. This could be due to the reaction dynamics of the primers used, although it is possible that with more extensive optimisation experiments, our PCR could have been made more sensitive. However, the inherent variability of biological samples suggests that such finely controlled experiments

may be of less value than the development of an overall optimal extraction method, by the use of an appropriate kit.

To increase sensitivity, nested PCR has been used in many studies of low abundance organisms (Gibbons and Awad-El-Kariem, 1999; Jiang et al., 1998; Saruta et al., 1997; Verdin et al., 2000); however, there is a high risk of contamination with primary PCR product in the nested PCR, and the use of a single step PCR is considered to provide increased specificity and reliability. In the primary step of a nested PCR, some amplification occurs in the presence of inhibitors, then some increased template in the form of primary products is available for the nested reaction. This two-step process results in the dilution of potential inhibitors and provision of sufficient template for the secondary reaction. Almost all samples that gave negative results in the primary PCR gave specific product after nested PCR, indicating the presence of bacterial DNA. We concluded that each method tested had extracted DNA from the samples, but not to a standard suitable for primary PCR, with the exception of kit 4.

The presence of PCR inhibitors in faecal samples would appear to vary between individuals, such as indicated by our results from samples H1 and H2. The extraction of amplifiable DNA from sample H2 was more successful than from sample H1 for most methods. Our sample size is too small to assess any possible gender effects. Diet is an important determinant of faecal composition and accordingly may influence the result of PCR on stool specimens. PCR inhibitors in faeces have been reduced in some individuals eating a diet devoid of plant material (Monteiro et al., 2001). Also, the moisture content of faeces could influence the concentration of inhibitors and the effective bacterial load added to the extraction process. However, faecal water content may not vary greatly between healthy individuals. Our samples included 50 mg wet weight of faeces, a lower amount than that used in some other studies (Holland et al., 2000), and accordingly may have improved the quality of the extractions by avoiding overload of the purification steps, albeit at the possible expense of some low-abundance bacteria. It is also possible that variation may exist between methods with respect to different bacterial species or population profile results, but we considered the two bacterial genera tested to be representative of major groups present in human faecal microflora. Similarly, faeces from other animals may have different properties in these extraction methods.

Without knowledge of the exact components in each of the kits, the reasons for the apparent advantage of kit 4 are speculative, although we noted the use of a polysaccharide mixture intended for the removal of inhibitors of the PCR reaction. Possible inhibitors include bile salts, degradation products of haemoglobin, and complex polysaccharides of plant origin (Monteiro et al., 1997; Widjojoatmodjo et al., 1992), but their exact inhibitory mechanisms remain unclear. Various additional procedures have been proposed to reduce PCR inhibitors, such as boiling the clinical sample in lysis buffer (Hubbard and Anderson, 1993; McOrist et al., 1994), or differential centrifugation steps for faeces (Wang et al., 1996), but these modifications were not fully evaluated for faeces and are unlikely to fully remove all potential inhibitors. In addition, these and other additional procedures are usually time consuming, expensive and of variable effect (Lantz et al., 1997; Monteiro et al., 1997; Makristathis et al., 1998). The addition of amplification facilitators such as bovine serum albumin is suggested to aid the amplification success for DNA from clinical samples (Al-Soud and Radstrom, 2000), but this was not evaluated here. Other parameters such as thermal cycler operation or *Taq* polymerase quality can have profound effects on the sensitivity of PCR reactions (for review see Tyler et al., 1997). It is worthy of note that our experimental endpoint was the production of PCR product and not the efficiency of DNA extraction. Furthermore, our experiments were conducted on faecal samples from only two individuals consuming a similar diet and do not represent a comprehensive analysis of human faecal samples and diet. However, our results illustrate the influence of DNA extraction method on experimental outcome, and that the use of appropriate DNA extraction kits or methods is a critical step for successful and valid PCR studies on clinical samples. Therefore, we recommend that DNA extraction techniques are carefully selected with particular regard to the specimen type. It seems to us that further comparative studies within and between laboratories are necessary to ensure that DNA extraction techniques are optimised for each particular application.

Acknowledgements

We thank Corinna Flory for skilled analytical work and technical support, and Anne McGuffin, Kay Pender and Manny Noakes for clinical trial support. We thank CSIRO for providing core funding for this project.

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